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ORIGINAL ARTICLE

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Long-term effects of fecal microbiota transplantation (FMT) in patients with irritable bowel syndrome

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Abstract

Background: We recently found fecal microbiota transplantation (FMT) in irritable bowel syndrome (IBS) patients to be an effective and safe treatment after 3 months. The present follow-up study investigated the efficacy and safety of FMT at 1 year after treatment.

Methods: This study included 77 of the 91 IBS patients who had responded to FMT in our previous study. Patients provided a fecal sample and completed five questionnaires to assess their symptoms and quality of life at 1 year after FMT. The dysbiosis index (DI) and fecal bacterial profile were analyzed using a 16S rRNA gene-based DNA probe hybridization. The levels of fecal short-chain fatty acids (SCFAs) were determined by gas chromatography.

Results: There was a persistent response to FMT at 1 year after treatment in 32 (86.5%) and 35 (87.5%) patients who received 30-g and 60-g FMT, respectively. In the 30-g FMT group, 12 (32.4%) and 8 (21.6%) patients showed complete remission at 1 year and 3 months, respectively; the corresponding numbers in the 60-g FMT group were 18 (45%) and 11 (27.5%), respectively. Abdominal symptoms and the quality of life were improved at 1 year compared with after 3 months. These findings were accompanied by comprehensive changes in the fecal bacterial profile and SCFAs.

Conclusions: Most of the IBS patients maintained a response at 1 year after FMT. Moreover, the improvements in symptoms and quality of life increased over time. Changes in DI, fecal bacterial profile and SCFAs were more comprehensive at 1 year than after 3 months. www.clinicaltrials.gov (NCT03822299).

KEYWORDS
fatigue, microbiome, short-chain fatty acids, superdonor, therapy

This study was previously presented at the UEG week virtual congress in October 2020.

1 | INTRODUCTION

Irritable bowel syndrome (IBS) is a common chronic gastrointestinal disorder, with IBS patients representing 12–14% of primary-care patient visits and 28% of referrals to gastroenterologists.[1-5] The current treatments used in the clinic focus on symptom relief. The intestinal bacterial profile of IBS patients deviates from that of healthy subjects, with a low diversity and or abnormal bacteria profiles (dysbiosis). However, there is disagreement regarding the distinct microbial signature of IBS patients.[6] The intestinal microbiota is considered to be one of the factors that plays crucial roles in the etiology of IBS.[1]

A recent randomized, double-blind, placebo-controlled study performed by our group found that fecal microbiota transplantation (FMT) was effective in improving the abdominal symptoms, fatigue, and quality of life in IBS patients.[7] These changes were accompanied by changes in the fecal bacterial profile.[7] However, these results were observed only at 3 months after FMT, and several questions remained to be answered, such as whether the clinical effect of FMT is sustained over the long term, whether the bacterial profile changes over time and whether there are any long-term adverse events. The present study therefore performed a 1-year follow-up of the responders to FMT in our previous study in order to answer these questions.

Key Points

- A recently published randomize controlled trial (RCT) from our group showed that fecal microbiota transplantation (FMT) improves IBS symptoms and the quality of life at 3 months after treatment. Mild self-limited adverse events were observed.
- The improvement in symptoms and quality of life in this RCT was accompanied marked changes in the fecal profiles of active treated patients.
- Most responders to FMT after 3 months maintain their response after 1 year. The effects of FMT on IBS symptoms, the quality of life, and the changes in the fecal bacteria increase over time. Furthermore, the microbial metabolism changed from a saccharolytic to a proteolytic fermentation pattern in IBS patients at 1 year after FMT.

2 | MATERIALS AND METHODS

2.1 | Study design

The design of this has been described in detail previously.[7] In brief, patients provided a fecal sample and completed five questionnaires to assess their symptoms and quality of life at baseline, and then provided another fecal sample and completed a new set of questionnaires at 1 month after FMT. They also completed an additional set of questionnaires at 3 months after FMT.[7] At 1 year after FMT, the patients completed another set of the questionnaires and provided a new fecal sample. The patients were asked to keep a diary to record bowel habits and register any adverse events. Polyethylene glycol and loperamide were allowed during the previous randomized, double-blind, placebo-controlled study as rescue medication. The patients were asked to continue using this rescue medication and to record their consumption. They were asked also to record the use of other IBS medication. The patients continued with their medication listed in Table 1 including proton-pump inhibitor (PPI).

2.2 | Patients

This study included 77 of the 91 IBS patients who responded to FMT in our previous study, defined as a decrease of ≥50 points in the IBS Severity Scoring System (IBS-SSS) total score.[7] The original 91 patients comprised 42 and 49 who received 30-g and 60-g FMT, respectively,[7] and 14 patients were either excluded or dropped out. Three patients were excluded because of pregnancy, one because of breast cancer diagnosis, three because of treatment with antibiotics for pharyngitis, pyelonephritis, and diverticulitis, one because of a bout of gastroenteritis caught abroad, and one because of abdominal surgery, while five patients dropped out. The remaining 77 patients comprised 37 and 40 who received 30-g and 60-g FMT. The characteristics of these patients at baseline are given in Table 1.

The inclusion and exclusion criteria of the present study are also provided in our previous report.[7] In summary, the inclusion criteria were being aged 18 to 85 years and having moderate-to-severe IBS symptoms, which were defined as an IBS-SSS total score of ≥175. The exclusion criteria were the presence of systemic disease, immune deficiency, or being treated by immune-modulating medication, pregnant, planning pregnancy, lactating, having a severe psychiatric disorder, having alcohol or drug abuse, or taking probiotics, antibiotics or IBS medications within 8 weeks prior to study inclusion.

2.3 | Donor characteristics and fecal sample collection, preparation, and administration

The single donor was a superdonor who has been described in detail previously.[7] Fecal samples were frozen immediately and kept at −20°C until they were delivered frozen to the laboratory, where they were kept at −80°C. The fecal bacterial and short-chain fatty acids analysis were performed on 63 fecal samples: 32 patients belonging to the 30-g group (28 responders and 4 non-responders) and 31 patients belonging to the 60-g group (27 responders and 4 non-responders). Fourteen fecal samples were discarded due to thawing during transport. The process of FMT has also been described in detail previously.[7] In brief, the patients who were randomized to receive FMT were divided into two groups: the first group received 30 g of donor feces and the second group received 60 g. The fecal

TABLE 1 Baseline characteristics of the included patients

	Overall	30-g FMT	60-g FMT
Number	77	37	40
Age, years	39.6 ± 11.6	39.6 ± 11.6	38.1 ± 14.4
Sex, female/male	62/15	29/8	33/7
IBS-D	32	17	15
IBS-C	25	11	14
IBS-M	20	9	11
IBS duration, years	15.5 ± 7.9	17.2 ± 9.3	14.7 ± 5.7
IBS-SSS score	311.3 ± 72.5	322.8 ± 64.1	315.3 ± 72.5
FAS score	31.3 ± 4.9	31.4 ± 5.1	31.2 ± 4.8
PPI medication	35 (45.5)	18 (48.6)	17 (42.5)
Birth-control medication	48 (62.3)	22(59.5)	26 (65.0)
Antimigraine medication	7 (9.1)	4 (10.8)	3 (7,5)
Medication against asthma/allergies	11 (14.3)	4 (10.8)	7(17.5)
Medication with levothyroxine	1 (1.3)	0 (0)	1 (2.5)
Medication with heart/vascular drugs	3 (3.9)	2 (5.4)	1 (2.5)

Note: Data are mean ± SD, *n*, or *n* (%) values.

Abbreviations: IBS-C, constipation-predominant IBS-C; IBS-D, diarrhea-predominant IBS; IBS-M, mixed-diarrhea-and-constipation IBS; PPI, proton-pump inhibitor.

material was thawed for 2 days at 4°C, mixed with 40 mL of sterile saline, filtered, and administered to the distal duodenum via the working channel of a gastroscope.

2.4 | Symptoms and quality of life assessment

Symptoms were assessed using the IBS-SSS and the Birmingham IBS Symptom Questionnaire, while fatigue was measured using the Fatigue Assessment Scale (FAS).[8,9] Patients who exhibited a decrease of ≥50 points in the IBS-SSS total score after FMT were considered responders, while those who had an IBS-SSS total score of ≤75 were considered to be in complete remission.[8] Quality of life was measured using the IBS Quality of Life (IBS-QoL) questionnaire and the Short-Form Nepean Dyspepsia Index (SF-NDI) questionnaire.[10-12]

2.5 | Fecal bacterial analysis

The fecal bacteria were analyzed using the GA-map Dysbiosis Test® method as described in detail previously.[13,14] In brief, the test uses the 16S rRNA gene to measure the fluorescence signals for 48 bacterial markers. This method was used for calculating both the bacterial profile and dysbiosis index (DI). The 48 bacterial markers detected bacteria within five phyla (Firmicutes, Proteobacteria, Bacteroidetes, Tenericutes, and Verrucomicrobia) that cover 10 bacterial classes, 36 genera, and 32 species.[13] This test assesses >300 bacteria at different taxonomic levels.[14] The DI was measured on a 5-point scale from 1 (normal) to five (severe dysbiosis), where DI values >2 indicate the presence of dysbiosis.[13]

2.6 | Determination of fecal levels of short-chain fatty acids

About 0.5 g of the fecal samples was homogenized with a solution containing 3 mmol/L 2-ethylbutyric acid (as internal standard) and 0.5 mmol/L H_2SO_4. A 2.5-mL aliquot of the homogenate was vacuum distilled, and the levels of short-chain fatty acids (SCFAs) were determined by gas chromatography (Agilent 7890 A, Agilent) using a capillary column (serial no. USE400345H, Agilent J&W GC columns, Agilent) and flame ionization detection.[15,16] The levels of total SCFAs and acetic, propionic, isobutyric, n-butyric, isovaleric, n-valeric acid, isocaproic, and n-caproic acids, with the results expressed in units of mmol kg^{-1} wet weight.

2.7 | Statistical analysis

The difference between the patients exhibiting complete remission at 3 months and 1 year after FMT was calculated using the chi-square test. Differences between patients at baseline, 3 months, and 1 year in the scores on the IBS-SSS, the Birmingham IBS Symptom, FAS, IBS-QoL, and SF-NDI questionnaires, and in the levels of SCFAs were analyzed using the Kruskal–Wallis test, with Dunn's multiple-comparisons test as a post-test. The Kruskal–Wallis test was also used to detect difference in the bacterial markers at baseline between responders, patients in remission, and patients who relapsed 1 year after FMT. The correlations between the changes in bacterial profiles and the IBS-SSS and FAS scores were analyzed using the non-parametric Spearman test and reported as correlation coefficient (r), *p* value and Bonferroni adjusted *p* values. Differences in

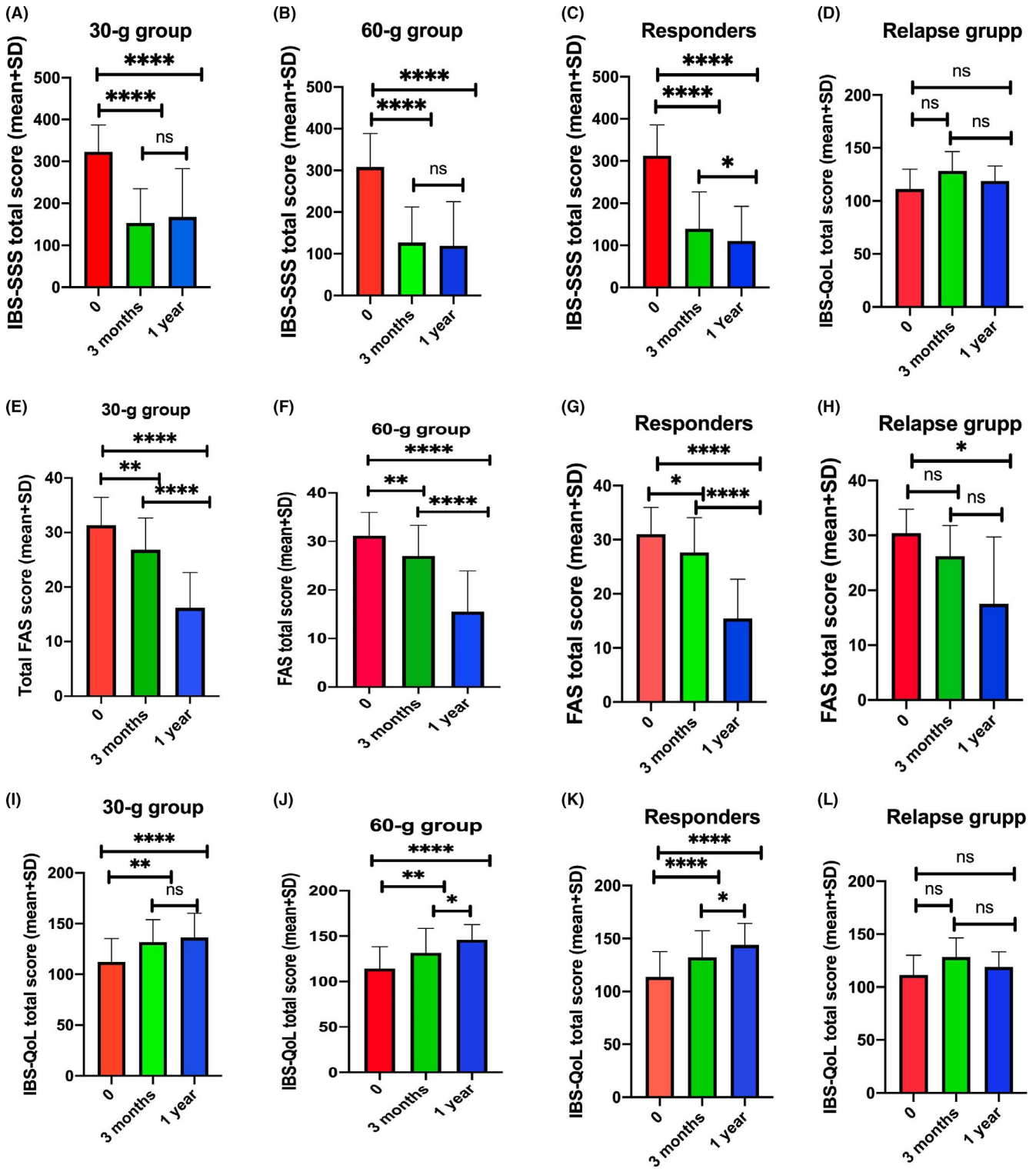

FIGURE 1 Total scores of IBS-SSS (A-D), of FAS (E-H) and of IBS-QoL (I-L) in the IBS patients who received 30 g transplant (30-g group), 60 g transplant (60-g group), responder (patients who exhibited a decrease of ≥50 points in the IBS-SSS total score 1 year after FMT) and relapse (patients who responded at 3 months, but did not respond 1 year after FMT) groups at baseline, at 3 months and 1 year after FMT. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{****}p < 0.0001$

DI score between baseline, 1 month, and 1 year were tested using the paired Wilcoxon test. The difference in fluorescence signals between baseline and 1 year after FMT was also tested using the paired Wilcoxon test. Adjusted *p* values were calculated using Bonferroni correction, with $p < 0.05$ considered significant. Scaled principal components analysis (PCA) of log-transformed fluorescence signals was performed, with the results visualized using different colors for different groups, together with ellipses that covered 80% of the

TABLE 2 IBS-SSS total score and the scores for its four subitems after FMTH

Time	Group	Total score	Subitem 1	Subitem 2	Subitem 3	Subitem 4
0	30-g FMT	322.8 ± 64.1	113.6 ± 39.2	55.0 ± 25.4	76.4 ± 19.2	75.6 ± 17.8
	60-g FMT	308.2 ± 79.8	106.5 ± 45.8	56.8 ± 27.2	80.0 ± 20.3	75.6 ± 20.8
3 months	30-g FMT	153.1 ± 82.1[a]	53.7 ± 38.9[a]	30.5 ± 21.7[a]	44.6 ± 29.7[a]	45.3 ± 30.9[a]
	60-g FMT	126.9 ± 95.5[a]	56.5 ± 47.5[a]	30.4 ± 26.4[a]	35.3 ± 27.5[a]	40.3 ± 27.5[a]
1 year	30-g FMT	167.0 ± 115.9[a]	59.0 ± 54.9[a]	27.3 ± 22.6[a]	45.1 ± 30.0[a]	41.5 ± 31.8[a]
	60-g FMT	119.4 ± 105.3[a]	41.7 ± 50.5[a]	19.4 ± 20.2[a]	30.0 ± 27.9[a]	30.9 ± 28.2[a]

Note: Data are mean ± SD values.

Subitems: 1, abdominal pain; 2, abdominal distension; 3, dissatisfaction with bowel habits; 4, interference with quality of life.

[a]$p < 0.0001$ compared with baseline.

TABLE 3 FAS scores in FMT-treated patients.

Time	Group	Total score	Physical fatigue	Mental health
0	30-g FMT	31.4 ± 5.1	16.0 ± 3.0	15.6 ± 2.8
	60-g FMT	31.2 ± 4.8	15.9 ± 2.8	15.5 ± 2.7
3 months	30-g FMT	26.8 ± 5.8[b]	13.3 ± 3.5	13.5 ± 3.0
	60-g FMT	27.0 ± 6.3[b]	14.0 ± 3.5[b]	13.0 ± 3.1[a]
1 year	30-g FMT	16.2 ± 6.5[c,d]	8.9 ± 3.9[c,d]	7.6 ± 3.3[c,d]
	60-g FMT	15.5 ± 8.4[c,d]	8.0 ± 4.5[c,d]	7.6 ± 4.0[c,d]

Note:: Data are mean±SD values.

[a]$p < 0.05$.

[b]$p < 0.01$.

[c]$p < 0.0001$ compared with baseline.

[d]$p < 0.0001$ compared with 3 months.

samples within each group. These analyses were performed using GraphPad Prism software (version 8.4.1, La Jolla) and R (version 3.6.3, R Foundation for Statistical Computing).

2.8 | Ethics

The study was approved by the Regional Committee for Medical and Health Research Ethics West, Bergen, Norway (approval no. 2017/1197/REK vest). All subjects provided both oral and written consents to participate. The study was registered at www.clinicaltrials.gov (NCT03822299).

All authors had access to the study data and reviewed and approved the final version of the manuscript.

3 | RESULTS

3.1 | Patient responses and use of medications

In the 30-g FMT group, 5 (13.5%) patients relapsed and 32 (86.5%) maintained their response to FMT. Twelve (32.4%) patients showed complete remission at 1 year, compared with 8 (21.6%) after 3 months ($p = 0.4$). In the 60-g FMT group, 5 (12.5%) patients relapsed and 35 (87.5%) continued to respond for FMT. Eighteen (45%) patients were in complete remission, compared with 11 (27.5%) after 3 months ($p = 0.1$).

All the relapsed patients (n = 10) used regularly medications. Four patients used loperamide, two patients used polyethylene glycol, and two patients used both polyethylene glycol and loperamide. The remaining two patients used linaclotide and prucalopride. In the patients that maintained response, two patients used loperamide only once during the following period and one patient used polyethylene glycol twice.

3.2 | Abdominal symptoms, fatigue, and quality of life

The maintained response in females was 90.3% and in males was 86.7% and the complete remission rate was 40.3% in females and 33.3% in males. The response rate and the complete remission rates did not differ significantly between females and males ($p = 0.6$ and 0.8, respectively). The maintained response rates were 90.6%, 88.0%, and 90.0% in IBS-D, IBS-C, and IBS-M patients, respectively. The complete remission rates were 41%, 40%, and 30% in IBS-D, IBS-C, and IBS-M patients, respectively. There was no significant difference between the IBS sub-types regarding either response or complete remission ($p = 0.9$ and 0.7, respectively).

The IBS-SSS total score and the scores for its four subitems were significantly lower than at baseline in both 30-g and 60-g groups. There were no significant differences in the IBS-SSS total scores or subitem scores between 3 months and 1 year after FMT (Figure 1 and Table 2). The IBS-SSS total score in responders was significantly lower at 1 year than at 3 months after FMT (Figure 1). In the patients who relapsed, while the IBS-SSS total score at the baseline was significantly higher than that at 3 months after FMT, it did not differ from that at 1 year after FMT (Figure 1). The IBS-SSS total scores were 135.9 ± 109.9, 141.2 ± 126.2, and 162.0 ± 102.5 in IBS-D, IBS-C, and IBS-M, respectively. The IBS-SSS total score did not differ significantly between the IBs sub-types ($p = 0.5$).

The total score for the Birmingham IBS Symptom Questionnaire and the scores in its three domains were significantly lower than those at baseline (Table S1). There was no difference in the

TABLE 4 IBS-QoL total scores and scores in its eight domains in FMT-treated patients

Time	Group	Total score	1	2	3	4	5	6	7	8
0	30-g FMT	12.2 ± 23.1	26.2 ± 6.9	20.0 ± 6.0	11.2 ± 3.4	10.1 ± 2.8	5.9 ± 2.8	13.6 ± 3.4	7.5 ± 2.1	10.8 ± 2.6
	60-g FMT	114.2 ± 24.0	27.4 ± 6.7	21.4 ± 5.5	11.5 ± 2.7	10.5 ± 2.7	6.7 ± 2.8	14.1 ± 3.4	7.7 ± 1.6	10.8 ± 2.8
3 months	30-g FMT	131.5 ± 22[b]	32.1 ± 6.9[d]	24.2 ± 5.2[c]	14.0 ± 3.0[c]	12.6 ± 2.9[d]	8.8 ± 2.8[d]	16.1 ± 2.6[c]	8.2 ± 1.6	12.6 ± 1.8[c]
	60-g FMT	131.5 ± 27.1[b]	32.2 ± 6.7[d]	24.0 ± 4.7[b]	14.3 ± 3.7[d]	12.7 ± 2.4	9.6 ± 3.6[d]	15.7 ± 3.4[a]	8.3 ± 1.6	12.1 ± 2.6[a]
1 year	30-g FMT	136.0 ± 24.0[d]	33.8 ± 5.9[d]	24.0 ± 5.0[b]	14.7 ± 3.2[d]	16.7 ± 3.0[d, g]	9.2 ± 3.7[d]	16.5 ± 3.3[c]	8.5 ± 1.7[a]	12.7 ± 2.1[c]
	60-g FMT	145.71 ± 16.9[d, e],	35.0 ± 4.9[d]	26.3 ± 3.4[d, e],	15.9 ± 2.8[d]	17.4 ± 2.3[d, h],	11.3 ± 2.8[d, g],	17.6 ± 1.9[d, e],	9.3 ± 1.1[d, f],	13.2 ± 1.7[d]

Note: Data are mean ± SD values. IBS-QoL domains: 1, dysphoria; 2, interference with daily activities; 3, body image; 4, health worries; 5,food avoidance; 6, social reaction; 7, sexual function; 8, impact on relationships.

[a] $p < 0.05$,
[b] $p < 0.01$,
[c] $p < 0.001$,
[d] $p < 0.0001$ compared with placebo.
[e] $p < 0.05$,
[f] $p < 0.01$,
[g] $p < 0.001$,
[h] $p < 0.0001$ compared with 30-g FMT.

Birmingham IBS Symptom Questionnaire total score or its three domain scores between 3 months and 1 year after FMT in both the 30-g and 60-g groups (Table S1).

In both the 30-g and 60-g FMT groups, the FAS score was significantly lower at 1 year after FMT than at baseline (Figure 1 and Table 3). The FAS scores in both the 30-g and 60-g FMT groups were significantly lower at 1 year than at 3 months after FMT. Similar results were observed in the responders. In the group exhibiting clinical relapse, the FAS score was significantly lower at 1 year after FMT than at baseline.

The IBS-QoL total scores increased significantly at 1 year after FMT in the 30-g FMT group, 60-g FMT group, and responders compared with those at baseline (Figure 1 and Table 4). In addition, the IBS-QoL total score was significantly higher at 1 year than at 3 months after FMT. In the patients who relapsed at 1 year after FMT, there was no significant improvement in the IBS-QoL at either at 3 months or 1 year after FMT. The deterioration in the quality of life as measured by the SF-NDI was significantly reduced at 1 year after FMT in both the 30-g and 60-g FMT groups (Table S2).

3.3 | Bacterial analysis

The median DI for all patients who received FMT was 3 (range: 1–5) at baseline and 2 (range: 1–4) at 1 year after FMT ($p < 0.0001$). In the 30-g FMT group, the median DI was 3 (range: 1–5) at baseline and 1 (range: 1–4) at 1 year after FMT ($p = 0.004$). In the 60-g FMT group, the median DI was 3 (range: 1–5) at baseline and 2 (range: 1–4) at 1 year after FMT ($p = 0.003$). The median DIs in the patients in complete remission were 3 (range: 1–5) and 2 (range: 1–3) at baseline and 1 year after FMT, respectively ($p = 0.01$); the corresponding values were 2 (range: 1–5) and 2 (range: 1–4), respectively ($p = 0.004$), in responders, and 3 (range: 1–3) and 2 (range: 1–3) ($p = 0.12$) in patients who relapsed.

There was a marked change in the bacterial profiles at 1 year after FMT compared to baseline in all patients who received FMT as well as in the 30-g and 60-g FMT groups, as detected by PCA and by analyzing differences in fluorescence signals (Figure 2).

The levels of 10 bacteria groups differed significantly between baseline and 1 year after FMT in patients who received FMT (Table 5). In the 30-g FMT group, the levels of three bacteria were significantly increased at 1 year after FMT: *Bacteroides stercoris*, *Alistipes* spp., and *Bacteroides* spp. & *Prevotella* spp. The levels of these bacteria were correlated inversely with both the IBS-SSS and FAS total scores (Table S3). The levels of *Eubacterium biforme* and *Parabacteroides* spp. also increased significantly at 1 year after FMT, but they were not correlated with either the IBS-SSS or FAS total score (Table S3).

In the 60-g FMT group ($N = 31$), the levels of *Bacteroides Spp. & Prevotella spp*, *Alistipes* spp., *Bacteroides stercoris*, *Parabacteroides* spp., and *Bacteroides zoogleoformans* increased at 1 year after FMT compared to the baseline sample and were inversely correlated correlate with both the IBS-SSS and FAS total scores, with the exception

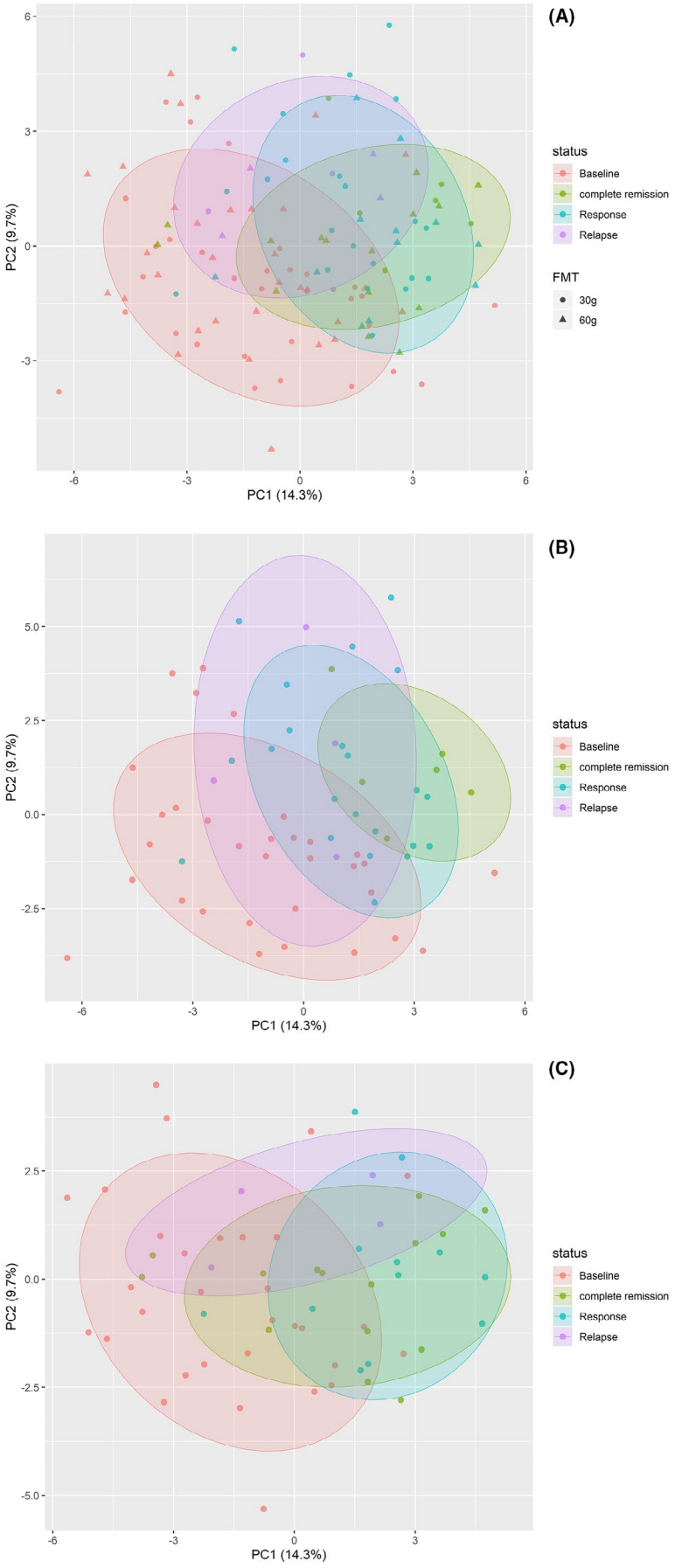

FIGURE 2 PCA plots of all FMT-treated patients (A), 30-g FMT group (B) and 60-g FMT group (C) at 1 year after FMT. The bacterial profile is indicated in orange for baseline, green for complete remission, purple for responders, and blue for relapses. Bacteria in the patients who received 30-g and 60-g FMT are indicated using circles and triangles, respectively. Ellipses of different colors enclose approximately 80% of the samples within the different groups. The bacterial profiles of the FMT-treated patients are grouped more to the right along the first PCA axis

TABLE 5 Bacteria whose levels differed significantly between baseline and 1 year after FMT in all patients who received FMT

Prob bacteria markers	Baseline	1 year after FMT	*p*	Adjusted *p*
206 Bacteroides spp. & *Prevotella* spp.	334 (9–1349)	944 (106–1330)	<0.001	<0.001
Alistipes spp.	43 (2–512)	228 (2–512)	<0.001	<0.001
207 Bacteroides stercoris	9 (2–69)	26 (2–212)	<0.001	<0.001
327 Streptococcus salivarius ssp. *thermophilus*	84 (5–907)	22 (2–732)	<0.001	<0.001
210 Parabacteroides spp.	6 (2–137)	38 (2–248)	<0.001	<0.001
208 Bacteroides zoogleoformans	20 (2–137)	45 (2–248)	<0.001	0.001
404 Shigella spp. & *Echerichia* spp.	20 (2–1167)	2 (2–725)	<0.001	0.002
314 Eubacterium hallii	151 (8–466)	102 (41–777)	<0.001	0.006
300 Firmicutes spp.	468 (68–1080)	677 (143–1304)	<0.001	0.016
500 Proteobacteria spp.	45 (16–1306)	29 (11–376)	<0.001	0.038

Note: Data are median (range) values.

of *Bacteroides zoogleoformans* (Table S4). On the other hand, the levels of *Streptococcus salivarius* ssp. *thermophilus* and *Eubacterium hallii* were decreased, and correlated significantly with the IBS-SSS and FAS total scores (Table S4).

In responders, the levels of nine bacteria were changed at 1 year after FMT (Table S5). The levels of these bacteria were correlated significantly with the IBS-SSS and FAS total scores, with the exceptions of *Parabacteroides* spp., *Shigella* spp. & *Escherichia* spp., and *Dorea* spp.

In patients exhibiting complete remission at 1 year after FMT, the levels of the following bacteria were significantly increased: *Bacteroides* spp. & *Prevotella* spp., *Alistipes* spp., and *Bacteroides stercoris*. In contrast, those of *Streptococcus salivarius* ssp. *thermophilus* and *Eubacterium hallii* were significantly decreased (Table S6).

No bacterial markers were significantly changed in the group of patients who had clinically relapsed at 1 year after FMT.

The only bacteria that differed at baseline between relapsed patients, patients in remission and responders were *Alistipes* spp. The level of *Alistipes* spp. was significantly lower in the relapsed patients than in the patients with remission and responders ($p = 0.008$, adjusted $p = 0.15$) (Figure S1).

3.4 | Fecal SCFA levels

In both the 30-g and 60-g FMT groups, the fecal levels of isobutyric and isovaleric acids increased at 1 year after FMT (Table S7). In the 60-g FMT group, the fecal level of total SCFAs increased at 1 year after FMT, whereas that of acetic acid decreased (Table S7). In the patients exhibiting complete remission and responders, the fecal levels of isobutyric and isovaleric acids increased at 1 year after FMT, whereas those of acetic and propionic acids decreased (Table 6). Moreover, in the patients exhibiting complete remission, the fecal levels of total SCFAs and butyric acid increased (Table 6). In the relapsed patients, the levels of fecal isobutyric and isovaleric acids increased at 1 year after FMT.

3.5 | Adverse events

Apart from the mild intermittent abdominal pain, diarrhea and constipation occurring at the first 2 days after FMT,[7] no adverse events were reported under the follow-up period. The 2 patients developed diverticulitis at 2 and 3 months after FMT reported previously did not have new diverticulitis attacks.[7]

4 | DISCUSSION

The present findings indicate that most of the patients who responded 3 months after FMT remained responders after 1 year.[7] Furthermore, there were more patients exhibiting clinically complete remission at 1 year after FMT than after 3 months.[7] Moreover, the abdominal symptoms and fatigue were significantly less severe and the quality of life was significantly better at 1 year than at 3 months after FMT.[7] Thus, it seems that the effect of FMT intensified in those who remained responding after 1 year. The finding that FMT induced remission in about half of the patients with IBS emphasizes the importance of the intestinal microbiota as an etiological factor of IBS.

Similar to our previous observation 3 months after FMT,[7] there was no difference between IBS sub-types regarding the response rate or IBS symptoms 1 year following FMT. In contrast to the recently published observation,[17] the response rate and IBS symptoms did not differ between females and males in the present study.

The DI did not improve at 1 month after FMT in our previous study,[7] whereas it had improved at 1 year after FMT in the present study. This indicates that the intestinal bacterial diversity had

TABLE 6 The SCFAs concentration in the feces of the remission, responders, and relapse groups at the baseline and 1 year after FMT

	Remission group			Responders			Relapse group		
Acid	**Baseline**	**1 year After FMT**	***p* values**	**Baseline**	**1 year After FMT**	***p* values**	**Baseline**	**1 year After FMT**	***p* values**
Total SCFA	74.5 ± 49.3	111-0 ± 23.0	0.008	71.5 ± 37.1	78.0 ± 42.1	0.5	69.6 ± 18.2	77.3 ± 26.6	0.6
Acetic acid	44.1 ± 21.4	31.7 ± 11.4	0.04	44.4 ± 21.3	37.5 ± 15.4	0.04	39.8 ± 10.4	40.3 ± 16.2	0.9
Propionic acid	14.8 ± 12.1	8.7 ± 4.5	0.02	13.2 ± 6.7	10.4 ± 5.9	0.03	11.9 ± 6.0	14.2 ± 7.1	0.6
Iso-butyric acid	1.2 ± 0.5	1.7 ± 0.7	0.03	1.3 ± 0.8	1.7 ± 1.8	0.002	1.0 ± 0.5	1.7 ± 0.6	0.03
Butyric acid	10.3 ± 7.6	13.6 ± 7.5	0.03	12.0 ± 9.1	10.3 ± 6.8	0.7	7.4 ± 3.1	11.3 ± 5.0	0.04
Iso-valeric acid	2.1 ± 1.5	2.9 ± 1.6	0.04	2.1 ± 1.4	2.4 ± 1.3	0.02	1.5 ± 0.9	2.3 ± 1.0	0.1
Valeric acid	1.4 ± 0.5	1.6 ± 0.6	0.6	1.8 ± 1.6	1.8 ± 0.9	0.3	1.1 ± 0.7	2.1 ± 0.6	0.01
Iso-capronic acid	0.018 ± 0.078	0.004 ± 0.021	0.5	0.010 ± 0.058	0.004 ± 0.21	0.9	0.000 ± 0.000	0.125 ± 0.315	0.5
Capronic acid	0.8 ± 1.0	0.6 ± 0.5	0.9	0.6 ± 0.5	0.6 ± 0.7	0.2	0.1 ± 0.3	0.4 ± 0.6	0.2

Note: The values were expressed as mmol kg^{-1} wet weight.

improved at 1 year after FMT. The changes in the bacterial profile in patients at 1 year after receiving FMT were more comprehensive than those we observed previously at 1 month after FMT, with 10 bacteria taxa changed after 1 year compared with 4 after 1 month.[7] In responders, the levels of nine bacteria taxa were significantly changed at 1 year after FMT compared with those at baseline. The levels of six of these bacteria taxa were correlated with the IBS-SSS and FAS scores. *Alistipes* are strictly anaerobic gram-negative rods that are resistant to bile and have limited ability to ferment carbohydrates.[18] *Alistipes* spp. levels in the relapsed patients were significantly lower at baseline than in responders and patients in remission at 1 year after FMT. This suggests that the levels of *Alistipes* spp. can be used to predict the outcome of FMT. Furthermore, *Alistipes* spp. levels increased as early as 1 month after FMT and remain high in responders at 1 year after FMT.[7] Moreover, they were strongly correlated with the total scores for both the IBS-SSS and FAS. Together these findings suggest that *Alistipes* spp.— which belong to the phylum Bacteroidetes—play a central role in the improvements seen after FMT. Further investigations are needed to clarify such a role.

The fecal SCFAs in IBS patients differ from those in healthy subjects, and it has been suggested that this difference plays a role in the pathophysiology of IBS.[19] It has been reported recently that FMT increases the fecal SCFA levels in IBS patients at 1 month after FMT.[20] Moreover, fecal butyric acid levels increased at 1 month after FMT and were inversely correlated to IBS-SSS and FAS total score.[20] The present study found a dose-dependent effect of FMT on the fecal SCFAs profile, with increased levels of the branched SCFAs isobutyric and isovaleric acids, and a decreased level of the straight SCFA acetic acid. These changes were more pronounced in patients with a sustained symptomatic effect at 1 year of FMT than in patients who relapsed. These results suggest an overall shift of the microbial metabolism, from a saccharolytic to a proteolytic fermentation pattern, which is conceivably of pathophysiological relevance.[21] The reduction in acetic acid levels in responders and patients in the 60-g FMT group may be particularly relevant since acetic acid has been found to induce visceral hypersensitivity in rodents.[22] It is noteworthy that similar changes of the SCFAs profile have been demonstrated previously in patients with IBS following adherence to a low-FODMAP (fermentable oligo-, di-, monosaccharides, and polyols) diet, an intervention that is also associated with relief of IBS symptoms.[23] The changes observed here in the fecal SCFAs may be caused by either changes in diet or changes in the intestinal bacterial profile. However, since the patients did not change their diet and that comprehensive change in the intestinal bacterial profile occurred after FMT, it is more likely that the changes in SCFAs are caused by the changes in the bacterial profile following FMT.

There were no reported adverse events at 1 year after FMT, which means that this treatment can be considered to be safe. However, one should keep in mind that the patients included in this study did not have systemic disease, immune deficiency (or were receiving an immune-modulating medication), a severe psychiatric disorder, or alcohol or drug abuse.

The major limitation of this study is that it did not follow-up the placebo group in our previous RCT study. In an earlier study, about 33.3% of IBS patients become asymptomatic in the long term.[24] Assuming that this would be the same in the placebo group this would be much lower than that observed in the active-treated groups. However, this limitation makes it difficult to assess the response using FDA/EMA endpoints or the difference medications use between the placebo and active treated groups in the follow-up period. Other limitations of this study were that it investigated only a rather small proportion of the total fecal bacterial content and that the levels of SCFAs were measured in feces, which does not accurately reflect the colonic production of SCFAs, since that mainly occurs in the caecum. Moreover, the findings for SCFAs reported here should be interpreted with caution, since luminal SCFA levels result from a complex interplay between diet (i.e., input of substrates for microbial fermentation), host (i.e., intestinal absorption capacity and transit of both substrates and SCFAs) and microbes (i.e., fermentation capacity).

In conclusion, the present follow-up study found that most patients who had responded to FMT after 3 months maintained their response after 1 year. Furthermore, their abdominal symptoms, fatigue, and quality of life were improved at 1 year compared with at 3 months after FMT. Finally, the changes in the intestinal bacterial profile and fecal SCFAs were more comprehensive at 1 year compared with at 3 months after FMT.

CONFLICT OF INTEREST

The authors have nothing to disclose.

AUTHOR CONTRIBUTIONS

M.E.S. designed the study, obtained the funding, administered the study, recruited the patients, performed FMT, collected, analyzed and interpreted the data, and drafted the manuscript. A.B.K. and C.C. contributed to the design of the study, analyzed and interpreted the results of the microbiome analysis, and critically revised the manuscript for important intellectual content. J.V. contributed to the design of the study, interpreted the results for SCFAs and critically revised the manuscript for important intellectual content. J.G.H., O.H.G., and T.H. contributed to the design of the study and to the analysis and interpretation of the data, and critically revised the manuscript for important intellectual content.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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